Behavioral/Systems/Cognitive

Rewarding Effects of AMPA Administration into the Supramammillary or Posterior Hypothalamic Nuclei But Not the Ventral Tegmental Area

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We examined whether injections of the excitatory amino acid AMPA are rewarding when injected into the posterior hypothalamus and ventral tegmental area. Rats quickly learned to lever-press for infusions of AMPA into the supramammillary or posterior hypothalamic nuclei but failed to learn to lever-press for similar injections into the ventral tegmental areas. AMPA injections into the supramammillary nucleus, but not the ventral tegmental area, induced conditioned place preference. The rewarding effects of AMPA appear to be mediated by AMPA receptors, because coadministration of the AMPA antagonist CNQX blocked the rewarding effects of AMPA, and administration of the enantiomer *R*-AMPA did not mimic the rewarding effects. AMPA injections into the supramammillary nucleus, but not the ventral tegmental area, also increased extracellular dopamine concentrations in the nucleus accumbens. Pretreatment with the D_1 dopamine antagonist SCH 23390 [*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine] led to extinction of AMPA self-administration. These findings implicate posterior hypothalamic regions in reward function and suggest that reward mechanisms localized around the ventral tegmental area are more complex than has been assumed recently.

Key words: **reinforcement; intracranial self-administration; conditioned place preference; microdialysis; nucleus accumbens; dopamine D_1 receptor antagonist; SCH 23390; excitatory amino acid; glutamate**

Introduction

The discovery that rats learn to lever-press compulsively for electrical stimulation of the lateral hypothalamic area (Olds, 1956) led to the early suggestion that the bed nucleus of the lateral hypothalamus was a point of synaptic integration of reward signals and function (Olds, 1956; Olds and Olds, 1965). However, the fact that this behavior was disrupted by blockade of dopamine receptors in more rostral structures (Liebman and Butcher, 1973; Fouriezos et al., 1978) implicated lateral hypothalamic fibers of passage rather than lateral hypothalamic synapses in brain stimulation reward. Paired-pulse collision tests confirmed that fibers of passage are involved (Gallistel et al., 1981). Current thinking is that descending fibers carry the signal, directly or indirectly, to the dopaminergic cells of the ventral tegmental area (VTA) (Wise, 1980; Yeomans, 1982). The VTA–accumbens dopamine neurons are thought to serve as the next relay in reward function (Wise and Rompre, 1989; Ikemoto and Panksepp, 1999).

Whereas electrical stimulation of the brain is useful for localization of function to fibers of passage, chemical stimulation offers selectivity for synaptic junctions. To examine whether posterior hypothalamic or ventral tegmental synapses are involved in reward function, we tested for rewarding effects of the excitatory amino acid AMPA microinjected into these areas. Ventral tegmental dopamine neurons receive excitatory amino acid afferents and are excited by the direct application of AMPA (Wang and French, 1993; Karreman et al., 1996; Kretschmer, 1999) or the AMPA agonist quisqualate (Suaud-Chagny et al., 1992; Chergui et al., 1993). These findings raise the possibility that AMPA administration into the VTA might be rewarding through activation of the mesoaccumbens dopamine neurons. Indeed, previous studies have shown that VTA administration of agents that activate the dopamine neurons, such as opioids and cholinergic agents (Leone et al., 1991; Westerink et al., 1996), is rewarding (Bozarth and Wise, 1981; Devine and Wise, 1994; Zangen et al., 2002). The neurons in the posterior hypothalamus also receive extensive excitatory amino acid afferents (Gonzalo-Ruiz et al., 1999; Kiss et al., 2002) and respond to glutamate application (Shepard et al., 1988; Carre and Harley, 1991). Although not traditionally linked to reward function, this region has been linked to other motivational functions (Swanson, 2000). Unexpectedly, we found that although AMPA injections into the VTA were not rewarding, AMPA injections into the supramammillary or posterior hypothalamic nuclei were.

Materials and Methods

Subjects. A total of 119 male Wistar rats (250–350 gm at the time of surgery; Harlan Industries, Indianapolis, IN) were housed in groups of two in a colony room with a constant temperature (21°C) and kept on a

Received Dec. 4, 2003; revised May 5, 2004; accepted May 10, 2004.

This work was supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services. We thank Dr. Zhi-Bing You for his help with HPLC.

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DOI:10.1523/JNEUROSCI.5367-04.2004

 0270-6474/04/245758-08$15.00/0

reversed 12 hr light/dark cycle (lights on at 9 P.M.). After surgery, they were housed individually. Food and water were available *ad libitum*, except during testing. The animals were treated in accordance with the guidelines of the National Institutes of Health and the protocols approved by the Animal Care and Use Committee of the National Institute on Drug Abuse Intramural Research Program.

Surgery and histology. Stainless-steel guide cannulas (24 gauge) were implanted in rats under intraperitoneal sodium pentobarbital (31 mg/kg) and chloral hydrate (142 mg/kg) anesthesia. In each animal, a guide cannula was inserted at a 6° angle from the vertical toward the midline to a target 1.0 mm above one of the intended sites of injection: the supramammillary nucleus, medial mammillary nucleus, posterior hypothalamic nucleus, anterior tip of the VTA just dorsal to the supramammillary nucleus, or anterior or posterior VTA (see Fig. 1*A*). Stereotaxic coordinates were 4.3 mm posterior to bregma, 1.3 lateral to the midline, and 8.2 mm ventral to the skull surface (measured along the trajectory of the angled cannula) for the supramammillary nucleus; 4.0 posterior, 1.4 lateral, and 9.2 ventral for the medial mammillary nucleus; 3.5–3.8 posterior, 1.0–1.3 lateral, and 7.5–8.0 ventral for the posterior hypothalamic nucleus; 4.3 posterior, 1.7 lateral, and 7.8 ventral for the anterior tip of the VTA; 4.8 posterior, 1.6 lateral, and 7.8 ventral for anterior VTA placements; and 5.8 posterior, 1.3 lateral, and 7.8 ventral for posterior VTA placements. The incisor bar was set at 3.3 mm below the interaural line. Rats were killed after completing experimental procedures, and their brains were processed (Ikemoto, 2002) for the microscopic examination of injection cannula placements. The brains of the rats used in the microdialysis experiment (see below) were processed for TH immunohistochemistry (Ikemoto, 2002).

Drugs. The excitatory amino acid AMPA, the inactive enantiomer *R*-AMPA (Sigma-RBI, St. Louis, MO), and the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX; Tocris Cookson, Ellisville, MO) were used for intracranial treatments; these drugs were dissolved in artificial CSF consisting of (in mM): 148 NaCl, 2.7 KCl, 1.2 $CaCl_2$, and 0.85 $MgCl_2$, pH adjusted to 6.5–7.8. The dopamine D_1 antagonist *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 23390) hydrochloride (Sigma-RBI) was used for intraperitoneal treatments and dissolved in 0.9% saline.

Self-administration procedure and apparatus. For operant testing, each animal was placed in a 30 × 22 × 24 cm^3 chamber. The chamber was equipped with a lever (45 mm wide × 2 mm thick, protruding 19 mm from the wall), a tone speaker, and a cue light located just above the lever. The chamber was enclosed in a sound-attenuating box equipped with a ventilating fan as well as a camera monitor system to observe animals during testing. Each rat's 31 gauge injection cannula was connected by polyethylene tubing to a micropump (Ikemoto and Sharpe, 2001) hanging a few millimeters above the rat's head. Each pump consisted of a miniature step motor and a small plastic reservoir. When activated, the motor advanced a shaft into the reservoir, displacing drug solution into the brain via the injection cannula.

Self-administration of AMPA with multiple concentrations and sites. Unilateral guide cannulas were aimed at five regions: the supramammillary nucleus, posterior hypothalamic nucleus, anterior tip of the VTA just dorsal to the supramammillary nucleus, and anterior and posterior VTA. Ten additional rats received unilateral cannulas toward the region just dorsal to the posterior hypothalamus ($n = 4$) or just lateral to ($n = 3$) or just ventral to ($n = 3$) the supramammillary nucleus. Five to 7 d after surgery, each rat was placed in the operant chamber. In the chamber, a response on the lever caused an intracranial infusion (75 nl) over 5 sec and illuminated the cue light just above the lever for 5 sec. Each rat earned 0.1 mM AMPA, 0.3 mM AMPA, vehicle, and 0.01 mM AMPA, in this order, over four sessions. Sessions lasted for 90 min, or until 80 infusions were earned. After these initial sessions, supramammillary and posterior hypothalamic rats that self-administered AMPA were retested with 0.3 mM AMPA.

Self-administration of AMPA with repeated sessions. Twenty-three rats were given 0.1 mM AMPA in sessions 2–4, 0.3 mM AMPA in sessions 7–9, and vehicle in sessions 1, 5, 6, and 10. To minimize tissue damage at injection sites, the maximum infusions were limited to 60, and each infusion was followed by a 10 sec time-out period during which additional lever-presses did not have any experimental consequence. Each infusion (75 nl) was delivered over 5 sec in response to a lever-press. The lever-press also turned on a cue light and tone for 1 sec to facilitate contingency learning between lever-presses and drug delivery.

Neurochemical specificity. Thirteen rats were trained to self-administer AMPA into the supramammillary nucleus using the operant procedure described above. After two or three training sessions with AMPA, they received one or two of the following experimental procedures. For the *R*-AMPA experiment, vehicle, the inactive enantiomer *R*-AMPA, and AMPA were given successively in three consecutive sessions. For the CNQX experiment, 0.3 mM AMPA containing 0.7 mM CNQX or 0.3 mM AMPA alone were given either in session 1 or in session 3. Vehicle was given in session 2. The order of testing with AMPA plus CNQX and with AMPA alone was counterbalanced. For the dopamine antagonist experiment, six rats were pretreated with either the D_1 dopamine antagonist SCH 23390 (0.05 mg/kg, i.p.) or 0.9% saline (1 ml/kg, i.p.) 30 min before intracranial self-administration testing. Over three sessions, they received AMPA, vehicle, and AMPA, in this order. Half of them received SCH 23390 in the first session and saline in the last; the other half received these treatments in reversed order. They also received saline before vehicle session.

Dopamine microdialysis in the accumbens. Dialysates were continuously collected in 20 min intervals and immediately frozen until they were assayed for dopamine. Five baseline samples (100 min) were collected, after continuous perfusion of artificial CSF overnight. The next day, vehicle, 0.1 and 0.3 mM AMPA, in this order, were microinjected (500 nl volume) (Ikemoto, 2002) into either the VTA or the supramammillary nucleus. The vehicle and 0.1 mM AMPA injections were separated by 80 min. The 0.1 and 0.3 mM AMPA injections were separated by 100 min. Dopamine was measured with HPLC coupled to an ESA (Chelmsford, MA) Coulochem II Detector (model 5200) with a dual-electrode microdialysis cell and an ESA model 501 data station. Sample injection onto the column (3 μm particle size; 3 × 150 mm; Analytical MD-150; ESA) was made automatically through remote control of the autosampler (CMA 200). The mobile phase for catecholamine separation consists of 75 mM NaH_2PO_4, 1.5 mM 1-octanesulfonic acid, 10 M EDTA, and 7% acetonitrile, pH 3.0 adjusted with H_3PO_4. Dopamine was quantified on reducing electrodes (−250 mV). The detection limit was 0.2 nM in 10 μl samples.

Conditioned place preference. The place-conditioning chamber consisted of two compartments (21 × 21 × 28 cm^3) and an area (21 × 21 × 12.5 cm^3) connecting the compartments; a guillotine door separated each compartment from the connecting area. One compartment differed from the other by wall color (black vs white), floor type (net vs grid), and lighting; the amount of light was modulated in each compartment so that rats did not prefer one compartment to the other before place conditioning. In session 1, each rat was placed in the place-conditioning chamber for 15 min without any treatment; the rat had access to both compartments, and the time spent in each compartment was recorded. In sessions 2 and 3, each rat was confined to one compartment or the other and received 25 5 sec infusions, at 70 sec intervals, of the type given in the self-administration studies: 0.3 mM AMPA or vehicle (counterbalanced order across subjects). In session 4, each rat was placed in the chamber without any treatment and with access to both compartments; the time spent in each compartment was recorded for 15 min. Sessions were separated by 24 hr.

A two-way mixed ANOVA was performed on place-preference time (the time spent in the AMPA-paired compartment minus the time spent in the vehicle-paired compartment) with injection site (supramammillary nucleus, anterior VTA, and posterior VTA) and conditioning (preconditioning and postconditioning). Because of a significant site-by-conditioning interaction, a Newman–Keuls *post hoc* test was conducted to compare preconditioning and postconditioning scores in each site.

Results

Self-administration of AMPA into the posterior hypothalamus and VTA

Rats were placed in operant chambers in which lever responses were rewarded with infusions of AMPA. The animals receiving AMPA into the supramammillary nucleus learned quickly to self-

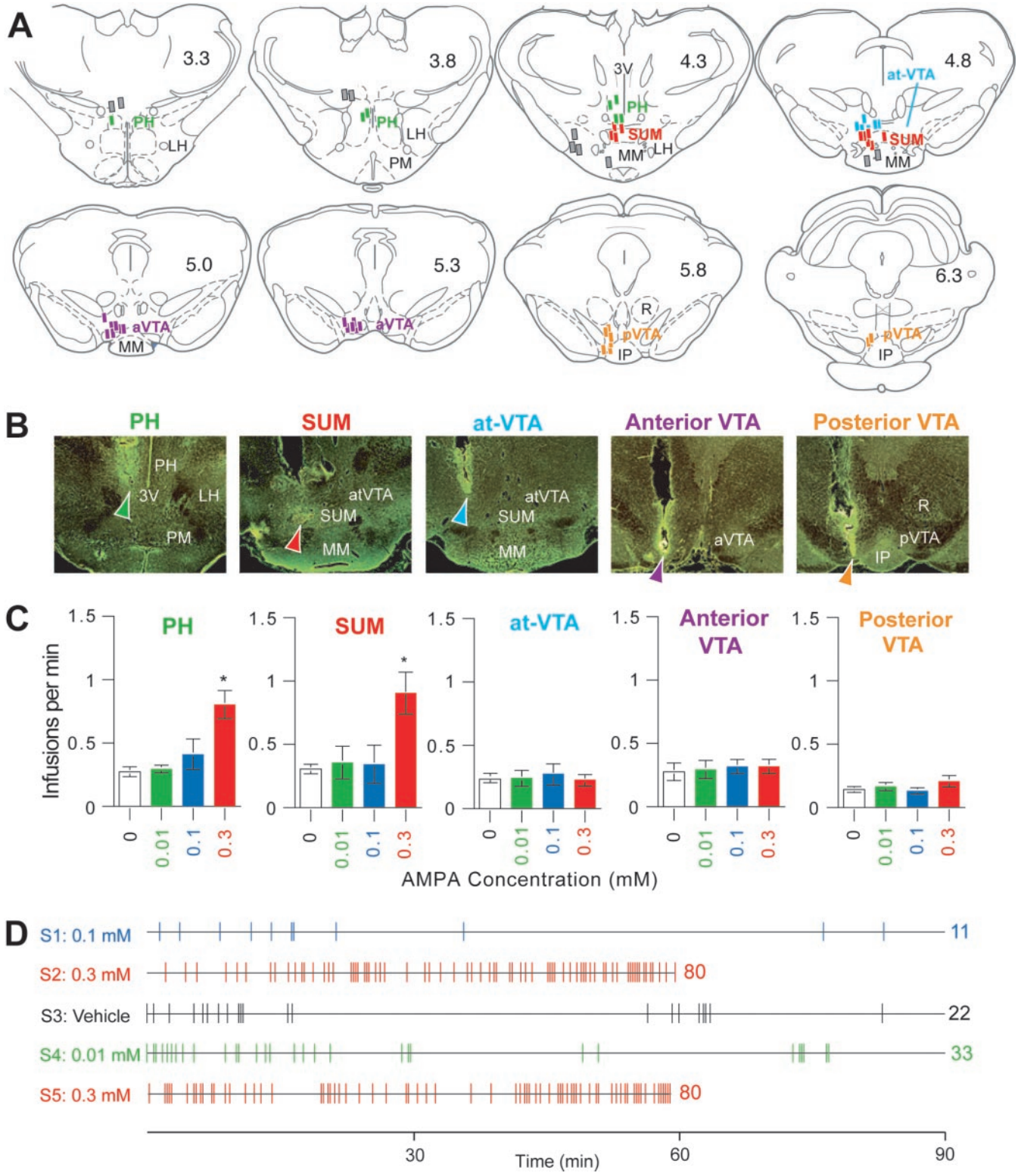

Figure 1. Rates and patterns of self-administration of AMPA as a function of injection site in and around the supramammillary nucleus (SUM). *A*, Sites of administration. Injection sites of each region are shown in rectangles with unique color. Placements shown in gray are outside of the regions analyzed statistically below. Drawings are adapted from the rat atlas of Paxinos and Watson (1997). 3V, Third ventricle; aVTA, anterior VTA; at-VTA, anterior tip of the VTA; IP, interpeduncular nucleus; LH, lateral hypothalamic area; MM, medial mammillary nucleus; PH, posterior hypothalamic nucleus; PM, premammillary nucleus; pVTA, posterior VTA; R, red nucleus. The numbers indicate distances (in millimeters) of sections posterior to bregma. *B*, Photomicrographs of brain sections stained with cresyl violet (inverted color). Arrowheads indicate the tips of typical injection cannulas. *C*, Mean ± SEM self-administration rates. *$p < 0.05$, significant difference from respective vehicle treatment with a Student–Newman–Keuls test. *D*, Event records of a representative animal receiving AMPA solutions into the SUM. Each of the five sessions (S1–S5) lasted for 90 min or until the rat self-administered 80 infusions. Horizontal lines indicate the durations of each session. Each vertical line indicates time of a self-infusion. The total numbers of infusions self-administered is indicated to the right of each trace.

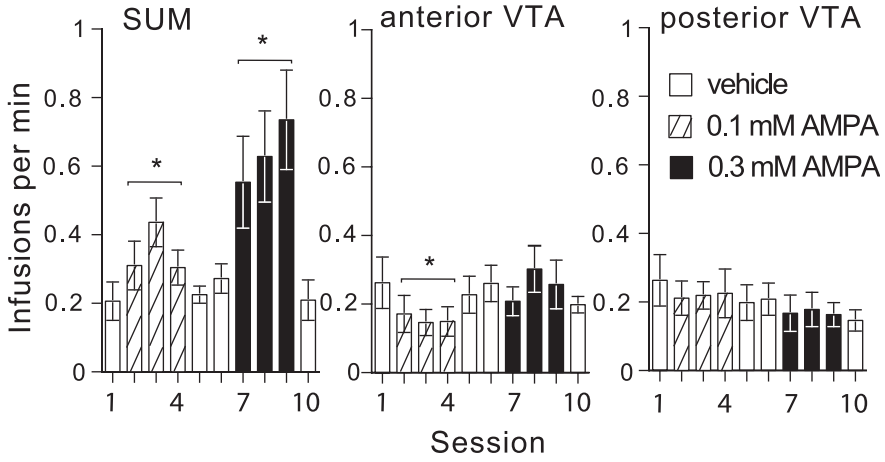

Figure 2. AMPA self-administration across repeated training sessions. Animals self-administered 0.1 mM AMPA less than vehicle into the anterior VTA. In contrast, the animals self-administered AMPA (0.1 and 0.3 mM) into the supramammillary nucleus (SUM) significantly more than vehicle. Data are mean ± SEM self-infusion rates. *$p < 0.05$, significant difference from respective vehicle treatments.

administer the substance (Fig. 1*C*). They self-administered 0.3 mM AMPA more avidly than they self-administered vehicle or lower concentrations of AMPA ($n = 11$; significant differences, $p < 0.05$, with Student–Newman–Keuls test following a significant main concentration effect in a one-way ANOVA; $F_{(3,30)} = 5.89$; $p < 0.005$). Data from a typical rat are shown in Figure 1*D*. Responding was minimal and intermittent when the rats were first tested with 0.1 mM AMPA in session 1. When they received 0.3 mM AMPA in the second session, they responded steadily and rapidly throughout their sessions, often obtaining the maximum 80 infusions within the 90 min test period. When receiving vehicle in session 3 or 0.01 mM AMPA in session 4, they tended to respond briefly, with intermittent returns to the lever. When 0.3 mM AMPA was reintroduced in session 5, the rats again self-administered it as avidly as in session 2 (Fig. 1*D*). Injections of AMPA into the posterior hypothalamic nucleus were similarly self-administered (Fig. 1*C*) ($n = 7$). The self-administration rates for 0.3 mM AMPA were again higher than those for vehicle or for other concentrations of AMPA (significant differences, $p < 0.05$, with Student–Newman–Keuls test following a significant main concentration effect in a one-way ANOVA; $F_{(3,18)} = 8.39$; $p < 0.005$).

The sites in this region where AMPA triggers rewarding actions appeared to be confined in the supramammillary and posterior hypothalamic nuclei. Four rats that had injection sites just dorsal to the posterior hypothalamic nucleus did not self-administer AMPA at any concentration (data not shown). Three rats with injection sites in the medial mammillary nucleus, just ventral to the supramammillary nucleus, and three rats with sites in the lateral hypothalamic area, just lateral to the supramammillary nucleus, also failed to reliably self-administer any concentration of AMPA (data not shown).

We also studied the effects of AMPA injections into the VTA, a heterogeneous area located just dorsal to and caudal to the supramammillary nucleus (Fig. 1*A*). We distinguished between the anterior and posterior portions of the VTA, because these regions are differentially responsive to several substances (Ikemoto et al., 1997, 1998; Carlezon et al., 2000; Rodd-Henricks et al., 2000; Ikemoto and Wise, 2002; Zangen et al., 2002; Bolanos et al., 2003). We also distinguished between the anterior tip, just dorsal to the supramammillary nucleus, and more posterior portions of the anterior VTA (Fig. 1*A*) for two reasons. First, the anterior tip has unique anatomical features. Although the anterior tip projects heavily to limbic forebrain regions, a much higher percentage of such projection neurons are dopaminergic in the rest of the VTA (Swanson, 1982). Swanson (1982) excluded the anterior tip from his analysis of the VTA [Swanson (1982), his Table 3, notes]. Paxinos and Watson (1997) now label this region the lateral hypothalamic area, although they previously labeled it the VTA (Paxinos and Watson, 1986). Second, injections into the region just dorsal to the supramammillary nucleus would be an important injection-site control, because solutions microinjected into the brain tend to diffuse dorsally along the cannula shaft. Six rats receiving AMPA into the anterior tip of the VTA, just dorsal to the supramammillary nucleus, failed to self-administer the drug (Fig. 1*C*) (the main concentration effect in a one-way ANOVA was not significant for animals with placements in the anterior tip; $F_{(3,15)} = 0.31$; $p = 0.82$). Rats also did not self-administer AMPA injections into either the anterior or posterior VTA (Fig. 1*C*). The main effect of concentration in a one-way ANOVA was not significant in the anterior ($n = 13$; $F_{(3,36)} = 0.40$; $p = 0.75$) or posterior ($n = 8$; $F_{(3,21)} = 2.71$; $p = 0.07$) VTA. Interestingly, infusions of 0.3 mM AMPA into each VTA site induced ipsilateral turning. Although not quantified, we observed this phenomenon in 7 of the 13 rats that received AMPA into the anterior VTA and five of the eight rats that received AMPA into the posterior VTA during or after self-administration sessions.

Histological analysis confirmed respective injection sites of these animals (Fig. 1*B*). It should be noted that animals that had self-administered the maximum 80 infusions of 0.3 mM AMPA in multiple sessions had obvious tissue damage at their injection sites. In subsequent self-administration experiments, the maximum infusion number was reduced to 60 and a 10 sec time-out after every infusion was introduced to minimize such damage.

Second test of AMPA self-administration

Our initial experiment raised two key concerns, which led us to further evaluate AMPA self-administration into the VTA and supramammillary nucleus. First, when animals are given intravenous drug administration, they frequently take more than one session to learn the task. In our first experiment, the animals were given only one session each for three different concentrations of AMPA. Several authors (Carlezon et al., 2000; You et al., 2001; Xi and Stein, 2002; Harris and Aston-Jones, 2003) have suggested that glutamate may play an important role in VTA reward function; we wanted to ensure that we gave the animals sufficient experience to learn that the injections were rewarding. Second, significant self-administration occurred only when animals received the 0.3 mM concentration of AMPA into the supramammillary nucleus; we had expected graded dose effectiveness. Thus, we designed the follow-up experiment to more closely examine the effects of 0.1 mM and 0.3 mM AMPA injections into the supramammillary nucleus. Again, neither 0.1 nor 0.3 mM AMPA injections were self-administered into the anterior ($n = 6$) or posterior VTA ($n = 8$) (Fig. 2). Interestingly, when the animals earned 0.1 mM AMPA into the anterior VTA, they lever-pressed less than when receiving vehicle [a significant treatment effect in

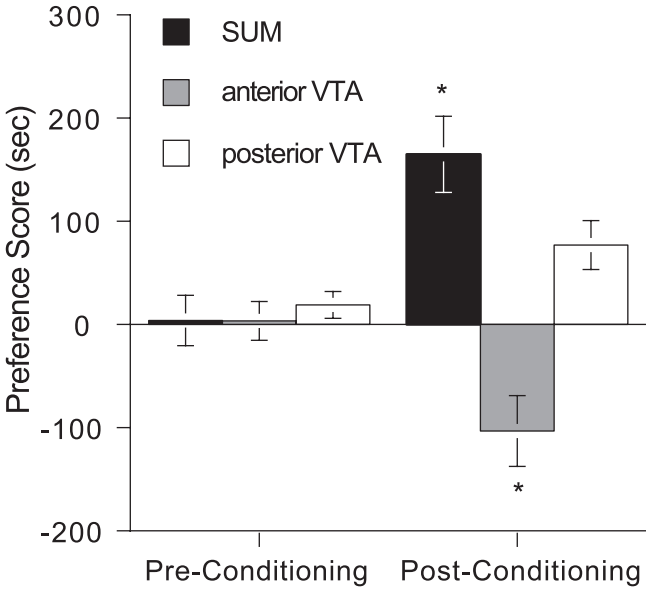

Figure 3. Time spent in the AMPA-paired compartment minus time spent in the vehicle-paired compartment (place-preference score). Conditioned place preference was induced by injections of AMPA into the supramammillary nucleus (SUM) but not the posterior VTA. Conditioned place avoidance was induced by AMPA injections into the anterior VTA. *$p < 0.05$, significant difference compared with place-preference time before conditioning.

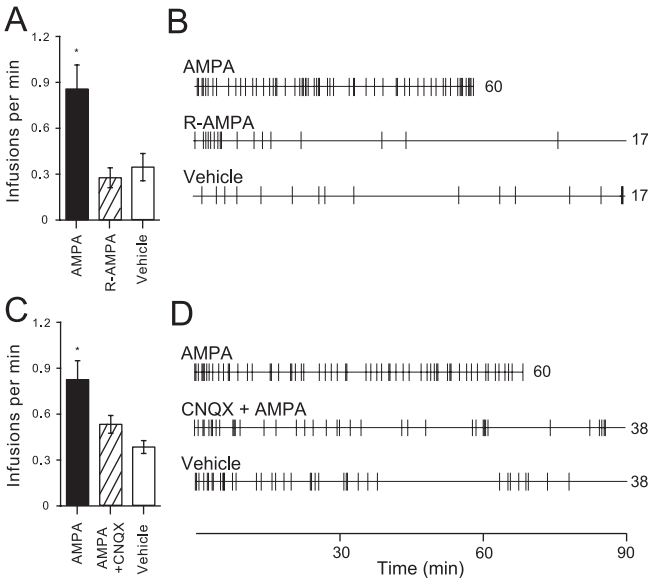

Figure 4. Neurochemical specificity of AMPA reward: tests with inactive enantiomer (*R*-AMPA) and with a receptor blocker (CNQX). *A, C*, Mean ± SEM infusion rates. *$p < 0.05$, significantly different from the other two treatments as revealed by a Student–Newman–Keuls test. *B, D*, Event records from representative rats. When *R*-AMPA was given, self-administration decreased markedly as the session progressed (*A, B*; $n = 7$). Self-administration of AMPA into the supramammillary nucleus was disrupted by coinfusion of the AMPA antagonist CNQX (*C, D*; $n = 6$). Horizontal lines indicate the durations of each session. Each vertical line indicates time of a self-infusion. The total numbers of infusions self-administered is indicated to the right of each trace.

a two-way ANOVA on infusion rates with three AMPA sessions (sessions 2–4) vs three vehicle sessions (sessions 1, 5, and 6); $F_{(1,5)} = 10.41$; $p < 0.05$]. Consistent with the first experiment, self-administration of 0.3 mM AMPA into the supramammillary nucleus was again greater than self-administration of vehicle [$n = 9$; a significant treatment effect in a two-way ANOVA with three AMPA sessions (sessions 7–9) vs three vehicle sessions (sessions 5, 6, and 10); $F_{(1,5)} = 12.33$; $p < 0.01$]. With the repeated training sessions, self-administration of 0.1 mM AMPA into the supramammillary nucleus also became greater than self-administration of vehicle [a significant treatment effect in a two-way ANOVA with three AMPA sessions (sessions 2–4) vs three vehicle sessions (sessions 1, 5, and 6); $F_{(1,5)} = 8.29$; $p < 0.05$].

Supramammillary AMPA induced conditioned place preference

To provide independent evidence regarding the apparent rewarding effects of supramammillary AMPA injections, we used a conditioned place-preference procedure. Nine rats received 0.3 mM AMPA into the supramammillary nucleus in one compartment of a place-preference apparatus and vehicle in the other compartment. In a subsequent drug-free session, they spent significantly more time in the compartment where they had received injections of AMPA into the supramammillary nucleus than in the compartment where they had received vehicle (Fig. 3) ($p < 0.01$). AMPA injections into the posterior VTA did not induce reliable conditioned place preference ($n = 10$; $p = 0.15$). Interestingly, AMPA injections into the anterior VTA induced significant conditioned place avoidance ($n = 7$; $p < 0.05$). The differential effect of AMPA injections as a function of injection sites is confirmed by a significant interaction effect between site and conditioning ($F_{(2,23)} = 11.5$; $p < 0.001$).

AMPA receptor mediation

To determine whether AMPA was rewarding because of its action at AMPA receptors, we examined the effects of the AMPA enantiomer *R*-AMPA and the AMPA antagonist CNQX. AMPA (0.3 mM) was again self-administered into the supramammillary nucleus, whereas 0.3 mM *R*-AMPA, which is inactive at AMPA receptors, was not self-administered (Fig. 4*A*) ($n = 7$; a significant main effect in a one-way ANOVA with infusion rates of vehicle, AMPA, and *R*-AMPA; $F_{(2,10)} = 14.46$; $p < 0.001$). The patterns of *R*-AMPA self-administration were not distinguishable from patterns of vehicle self-administration; rats mainly responded during the first 15–20 min (Fig. 4*B*). Moreover, when CNQX was coadministered with AMPA, rats responded less than when they received AMPA alone (Fig. 4*C*) ($n = 6$; a significant main effect in a one-way ANOVA with infusion rates of respective solutions; $F_{(2,10)} = 8.70$; $p < 0.01$). Like self-administration of vehicle, the rats frequently self-administered the mixture of AMPA and CNQX in the beginning of the session and self-administered very little later in the session (Fig. 4*D*).

Relationships between dopamine and rewarding effects of AMPA

VTA–accumbens dopamine neurons play an important role in the reward process. We therefore used microdialysis to determine whether supramammillary or ventral tegmental injections of AMPA would elevate nucleus accumbens dopamine levels. Supramammillary injections of AMPA increased extracellular dopamine in the accumbens (Fig. 5*A*) [$n = 9$; a significant treatment effect on percentage of baseline in a two-way ANOVA with three treatments (vehicle, 0.1, and 0.3 mM AMPA) and three 20 min blocks after each injection; $F_{(2,16)} = 4.25$; $p < 0.05$]. Injections of 0.3 mM AMPA were particularly effective ($p < 0.05$ with a Student–Newman–Keuls test). Injections of 0.1 or 0.3 mM AMPA into the posterior VTA, which is rich in dopaminergic neurons (Fig. 5*B*), were ineffective ($n = 8$; $F_{(2,14)} = 0.88$; $p = 0.43$).

We subsequently determined whether self-administration of

AMPA into the supramammillary nucleus is dependent on normal dopamine neurotransmission. Rats were initially trained to self-administer AMPA into the supramammillary nucleus. They were then pretreated with the D_1 dopamine antagonist SCH 23390 (0.05 mg/kg, i.p.) in one session and saline in another. When receiving the dopamine antagonist 30 min before AMPA self-administration, the animals self-administered AMPA at markedly lower rates than when receiving saline (Fig. 5*C*) ($n = 6$; a significant main effect in a one-way ANOVA with infusion rates of respective solutions; $F_{(2,10)} = 27.83$; $p < 0.0001$). The pattern of AMPA self-administration with SCH 23390 was similar to that of vehicle self-administration (Fig. 5*D*). Although treatment with the dopamine antagonist did not disrupt initiation of self-administration or increase initial infusion intervals, it markedly increased infusion intervals later in sessions (Table 1). This observation was confirmed by a significant treatment-by-interval interaction in a two-way ANOVA with the three treatments (AMPA with SCH 23390, AMPA with saline, and vehicle with saline) and two infusion intervals (first vs last) ($F_{(2,10)} = 6.24$; $p < 0.05$).

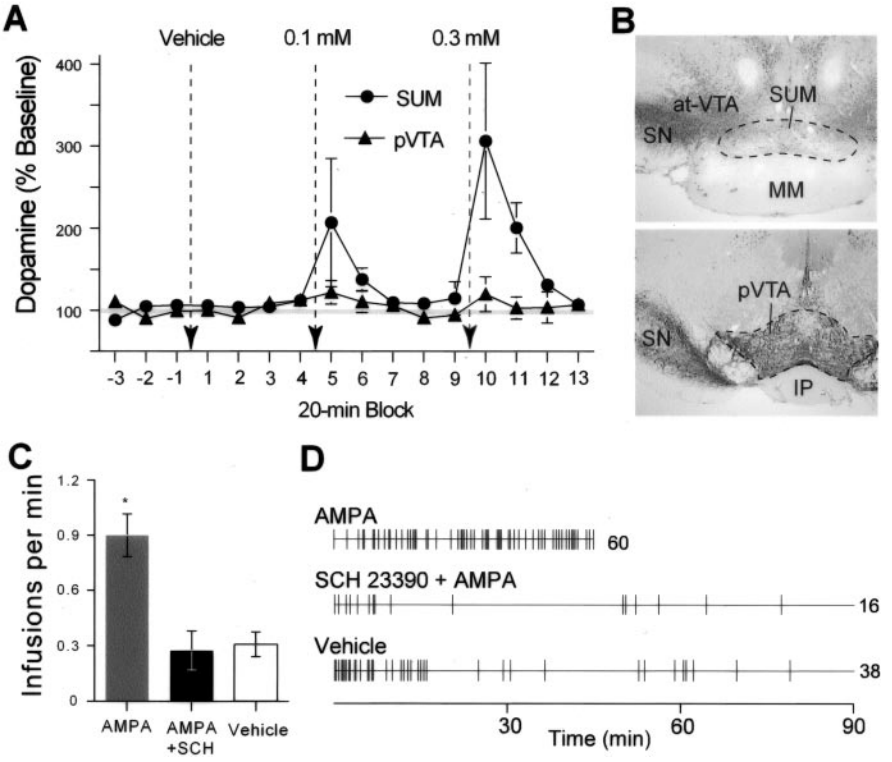

Figure 5. Dopamine and supramammillary AMPA reward. *A*, Dopamine release. Injections of 0.3 but not 0.1 mM AMPA (500 nl volume) into the supramammillary nucleus (SUM) significantly increased extracellular dopamine levels in the accumbens. However, injections of 0.1 or 0.3 mM AMPA into the posterior VTA (pVTA) did not significantly increase accumbal dopamine levels. Data are mean ± SEM percentage of baseline. *B*, Dopaminergic cells at the levels of the SUM and pVTA. Brain sections were immunostained for tyrosine hydroxylase. Dopaminergic cell bodies are densely aggregated in the VTA, whereas the SUM has very few dopaminergic cell bodies. SN, Substantia nigra; MM, medial mammillary nucleus; IP, interpeduncular nucleus; aVTA, anterior VTA. *C, D*, Effect of dopamine D_1 antagonist. Pretreatment of the D_1 antagonist SCH 23390 (0.05 mg/kg, i.p.) extinguished AMPA self-administration in the SUM. *$p < 0.05$, significant difference from vehicle or AMPA plus SCH. In *D*, horizontal lines indicate the durations of each session. Each vertical line indicates time of a self-infusion. The total numbers of infusions self-administered is indicated to the right of each trace.

Discussion

The absence of expected effects of AMPA in the VTA

Because activation of the mesoaccumbens dopamine system is rewarding, and because mesoaccumbens dopamine neurons are activated by AMPA application (Wang and French, 1993; Karreman et al., 1996; Kretschmer, 1999), we were surprised to find that AMPA was not self-administered into the VTA. Our initial finding was confirmed by a second self-administration experiment. A third experiment, in this case a conditioned place-preference procedure, again showed that AMPA administration into the VTA was not rewarding. Finally, we used microdialysis to reevaluate the idea that AMPA application into the VTA stimulates the mesoaccumbens dopamine system; AMPA administration into the VTA did not increase extracellular dopamine in the accumbens.

Table 1. Effects of SCH 23390 on the initiation of self-administration and first and last infusion intervals in seconds [mean (SEM)]

	Treatments: pretreatment plus infusate		
	SCH 23390 plus AMPA	Saline plus AMPA	Saline plus vehicle
Latency	59.7 (38.4)	35.5 (15.8)	19.0 (10.2)
First interval	48.3 (13.1)	50.3 (15.6)	59.2 (18.4)
Last interval	1186.5* (439.2)	35.3 (5.1)	469.7* (142.4)

*Significantly different from respective first infusion interval.

Our finding that VTA AMPA injections did not elevate dopamine levels in the nucleus accumbens led us to evaluate more closely previous studies on this topic. Kretschmer (1999) reported that ventral tegmental administration of AMPA (0.1 mM for 60 min via microdialysis probe) increased extracellular dopamine levels in the nucleus accumbens by 15%. Kretschmer's finding concerning dopamine in the accumbens seems consistent with the present finding that AMPA administration (0.1 mM in 500 nl) into the VTA increased dopamine levels by 20% (although the increase in our study was not statistically significant). Suaud-Chagny et al. (1992) determined dopamine levels in the accumbens using electrochemically treated electrodes and found that AMPA application into the VTA caused an increase in dopamine levels that lasted for only a few seconds. Because the study by Suaud-Chagny et al. (1992) involved different experimental procedures than the present and other microdialysis studies, they are difficult to directly compare. However, the comparison by Suaud-Chagny et al. (1992) of the effects of quisqualate with the effects of NMDA on accumbens dopamine levels suggest that the effects of quisqualate on accumbens dopamine levels are only half as large, in terms of the amplitude of the dopamine level, and one-third as long as the effects of NMDA. Thus, the findings of Suaud-Chagny et al. (1992) again suggest that the effects of VTA AMPA on nucleus accumbens dopamine are considerably weaker than those we found to accompany the behaviorally relevant injections of AMPA into other sites.

The third study of the effect of VTA AMPA on dopamine, a microdialysis study, involved probe placements that may be more relevant to the nearby supramammillary and posterior hypothalamic nuclei than to the dopaminergic cells of the VTA. Karreman et al. (1996) reported that AMPA administration (0.1 mM for 20 min via microdialysis probe) into the VTA increased ex-

tracellular dopamine levels in the accumbens by 100% from the baseline. However, the micrograph in the study by Karreman et al. (1996) shows a "VTA" probe placed in the anterior tip of the VTA and also shows the tip of the probe making contact with the supramammillary nucleus. It is not clear that drugs infused at this site should be attributed to actions in the VTA; reverse dialysis from this site would irrigate the supramammillary nucleus as well. In any case, the finding that our rats did not self-administer AMPA into the anterior tip of the VTA is consistent with the idea that AMPA acting within this zone does not stimulate reward circuitry.

Although studies performed with single-unit recording procedures suggest that the application of AMPA or the AMPA agonist quisqualate stimulates VTA dopamine neurons (Chergui et al., 1993; Wang and French, 1993), the mesoaccumbens dopamine neurons may not be activated after microinjections of AMPA. Microinjections of AMPA in this region are also likely to activate local GABAergic neurons that inhibit their dopaminergic neighbors (Johnson and North, 1992). Ventral tegmental GABAergic neurons, like dopaminergic neurons, receive excitatory amino acid afferents (Carr and Sesack, 2000), and nondopaminergic (putative GABA) neurons are stimulated by AMPA application (Wang and French, 1995). Therefore, ventral tegmental injections of AMPA will stimulate both dopaminergic and GABAergic neurons. The net effect of stimulating both dopaminergic and GABAergic neurons could lead to no change or even reduced activation of dopaminergic neurons. In any case, there is no substantial evidence that ventral tegmental microinjections of AMPA stimulate the mesoaccumbens dopamine system sufficiently to alter behavior.

AMPA administration into the supramammillary or posterior hypothalamic nuclei is rewarding

Previous studies suggest that direct electrical stimulation of the supramammillary or posterior hypothalamic region is rewarding (Olds and Olds, 1963). More recently, while studying the effects of injections into the VTA, we (Ikemoto et al., 1997) observed that rats self-administer $GABA_A$ receptor antagonists into the supramammillary nucleus. These observations do not necessarily suggest that these are synaptic sites of transmission of reward-related signals. The effects of electrical stimulation could be mediated by fibers of passage, and the effects of chemical administration could be mediated by the diffusion of the drug to neighboring regions.

The present study was designed to determine precisely the location of synaptic sites of chemical transmission of reward-related signals. The present site-efficacy experiment allows us to suggest that AMPA was rewarding because of receptor actions in the supramammillary nucleus and posterior hypothalamic nucleus. The rewarding effects of the posterior hypothalamic nucleus do not appear to be mediated by the diffusion of the drug into the supramammillary nucleus or vice versa, because the concentration–efficacy relationship of AMPA in the posterior hypothalamic nucleus is similar to that of AMPA in the supramammillary nucleus. We determined that the rewarding effects of AMPA were mediated by AMPA receptors, because the inactive enantiomer of AMPA was not self-administered and because the rewarding effects of AMPA were decreased by coadministration of the AMPA antagonist CNQX. The conditioned place preference induced by AMPA administration confirms that AMPA actions at this site are rewarding. Therefore, we suggest that AMPA receptors within the supramammillary and posterior hypothalamic nuclei mediate positive reinforcing effects of our injections.

Carlezon et al. (2000) found that rats selectively overexpressing the AMPA receptor subunit glutamate receptor 1 in the posterior VTA develop aversive responses to systemic morphine treatment, whereas rats receiving the viral vector into the anterior tip of the VTA or supramammillary nucleus show enhanced sensitivity to the rewarding effects of systemic morphine. Although it is not clear exactly how the findings by Carlezon et al. (2000) relate to the present findings, both studies suggest regional differences in the roles of AMPA receptor activation in the VTA and its neighboring regions.

Posterior hypothalamic AMPA reward and the mesoaccumbens dopamine system

Reward-related neurons in the supramammillary nucleus appear to be closely linked with the mesoaccumbens dopamine system. We found that AMPA administration into the supramammillary nucleus but not the VTA increased extracellular dopamine in the accumbens. In addition, pretreatment with the D_1 dopamine antagonist extinguished AMPA self-administration, suggesting that dopamine transmission is necessary for the rewarding effects of AMPA in this area.

Possible circuitry connecting the supramammillary nucleus with the mesoaccumbens dopamine system includes direct projections of supramammillary neurons to the VTA (Vertes, 1992) and indirect pathways from supramammillary neurons to brain regions that project directly to the VTA, including the septum, diagonal band, bed nucleus of the stria terminalis, substantia innominata, preoptic area, and lateral habenular nucleus (Vertes, 1992; Vertes et al., 1995). In addition, the hippocampus, which receives extensive afferents from the supramammillary nucleus, projects to the accumbens. Signals from the hippocampus may modulate dopamine release in the accumbens as well as effects of dopamine on medium spiny neurons of the accumbens.

Because of their role in positive reinforcement and their close link to the mesoaccumbens dopamine system, the supramammillary and posterior hypothalamic nuclei may also be hypothesized to play important roles in learning and motivated behaviors involving a variety of rewards, as shown for the VTA. This suggestion is consistent with the recent suggestion by Swanson (2000) that the medial portion of the posterior hypothalamic area, in addition to the VTA and substantia nigra, is involved in the expression of motivated behavior. The roles of these structures in natural rewards such as food and sexual interaction remain to be investigated.

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